

T, 1330

Table 1. Sequence of LoxP Sites

LoxP WT 5'-ATAACTTCGTATAATGTATGCTATACGAAGTTAT-3'
[SEQ ID NO: 1]

LoxP511 5'-ATAACTTCGTATAGTATACATTATACGAAGTTAT-3'
[SEQ ID NO: 2]

LoxC2 5'-ACAACTTCGTATAATGTATGCTATACGAAGTTAT-3'
[SEQ ID NO: 3]

LoxP1 5'-ATAACTTCGTATAATATATGCTATACGAAGTTAT-3'
[SEQ ID NO: 4]

LoxP2 5'-ATAACTTCGTATAGCATACATTATACGAAGTTAT-3'
[SEQ ID NO: 5]

LoxP3 5'-ATAACTTCGTATAATGTATACTATACGAAGTTAT-3'
[SEQ ID NO: 6]

LoxP4 5'-ATAACTTCGTATAATATAAACTATACGAAGTTAT-3'
[SEQ ID NO: 7]

LoxP5 5'-ATAACTTCGTATAATCTAACCTATACGAAGTTAT-3'
[SEQ ID NO: 8]

LoxP6 5'-ATAACTTCGTATAACATAGCCTATACGAAGTTAT-3'
[SEQ ID NO: 9]

LoxP7 5'-ATAACTTCGTATAACATACCCTATACGAAGTTAT-3'
[SEQ ID NO: 10]

LoxP8 5'-ATTACCTCGTATAGCATACATTATACGAAGTTAT-3'
[SEQ ID NO: 11]

LoxP9 5'-ATAACTTCGTATAGCATACATTATATGAAGTTAT-3'
[SEQ ID NO: 12]

LoxP10 5'-ATTACCTCGTATAGCATACATTATATGAAGTTAT-3'
[SEQ ID NO: 13]

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Table 2. Sequence of PCR primers for amplifying heavy- and light-chain genes of human antibody.

(B= C/G/T; D= A/G/T; K= G/T; M= A/C; R= A/G; S= C/G; W= A/T; and Y= C/T)

a) Heavy-chain VH

5'-primers (back primers):

VH1b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG CAG GAG
TCS G-3' [SEQ ID NO: 14]

VH2b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTA CAG CTG CAG CAG
TCA-3' [SEQ ID NO: 15]

VH3b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTA CAG CAG
TGG G-3' [SEQ ID NO: 16]

VH4b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC GAG GTG CAG CTG KTG GAG
WCY-3' [SEQ ID NO: 17]

VH5b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTC CAG CTK GTR CAG
TCT GG-3' [SEQ ID NO: 18]

VH6b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG RTC ACC TTG AAG GAG
TCT G-3' [SEQ ID NO: 19]

VH7b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG GTG SAR
TCT GG-3' [SEQ ID NO: 20]

3'-primers (forward primers):

VH1f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC
TCC GCC GCC TGA TCC ACC ACC GCC TGA GGA GAC RGT GAC CAG
GGT G-3' [SEQ ID NO: 21]

VH2f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC
TCC GCC GCC TGA TCC ACC ACC GCC TGA GGA GAC GGT GAC CAG
GGT T-3' [SEQ ID NO: 22]

VH3f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC
TCC GCC GCC TGA TCC ACC ACC GCC TGA AGA GAC GGT GAC CAT
TGT-3' [SEQ ID NO: 23]

VH4f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC
TCC GCC GCC TGA TCC ACC ACC GCC TGA GGA GAC GGT GAC CGT
GGT CC-3' [SEQ ID NO: 24]

VH5f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC
TCC GCC GCC TGA TCC ACC ACC GCC GGT TGG GGC GGA TGC ACT
CC-3' [SEQ ID NO: 25]

VH6f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC
TCC GCC GCC TGA TCC ACC ACC GCC SGA TGG GCC CTT GGT GGA
RGC-3' [SEQ ID NO: 26]

b) Light-chain Vλ

5'-primers (back primers):

Vλ1b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT CAG TCT GTS BTG ACG CAG
CCG CC-3' [SEQ ID NO: 27]

Vλ2b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT TCC TAT GWG CTG ACW CAG
CCA C-3' [SEQ ID NO: 28]

Vλ3b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT TCC TAT GAG CTG AYR CAG
CYA CC-3' [SEQ ID NO: 29]

Vλ4b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT CAG CCT GTG CTG ACT CAR
YC-3' [SEQ ID NO: 30]

Vλ5b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT CAG DCT GTG GTG ACY CAG
GAG CC-3' [SEQ ID NO: 31]

135

Vλ6b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT CAG CCW GKG CTG ACT CAG
CCM CC-3' [SEQ ID NO: 32]

Vλ7b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT TCC TCT GAG CTG AST CAG
GAS CC-3' [SEQ ID NO: 33]

Vλ8b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT CAG TCT GYY CTG AYT CAG
CCT-3' [SEQ ID NO: 34]

Vλ9b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT AAT TTT ATG CTG ACT CAG
CCC C-3' [SEQ ID NO: 35]

3'-primers (forward primers):

Vλ1f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TTC TAG GAC GGT SAS CTT GGT
CC-3' [SEQ ID NO: 36]

Vλ2f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TTC GAG GAC GGT CAG CTG GGT
GC-3' [SEQ ID NO: 37]

c) Light-chain Vκ

5'-primers (back primers):

Vκ1b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT GAC ATC CRG DTG ACC CAG
TCT CC-3' [SEQ ID NO: 38]

Vκ2b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT GAA ATT GTR WTG ACR CAG
TCT CC-3' [SEQ ID NO: 39]

Vκ3b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT GAT ATT GTG MTG ACB CAG
WCT CC-3' [SEQ ID NO: 40]

Abstract

Abstract

Abstract

Abstract

Abstract

Abstract

a) The sense strand

b) The antisense strand

5'-TCG ATT AAT TAA GCG CGC ACT GCC TCC ACC ACC GCT GCC
ACC TCC GCC AGA TCC TCC GCC GCC TGA TCC ACC ACC GCC-3'
[SEQ ID NO: 47]

Abstract

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Table 4. Sequence of PCR primers that include loxP sites for amplifying heavy- and light-chain genes of human antibody.

(B= C/G/T; D= A/G/T; K= G/T; M= A/C; R= A/G; S= C/G; W= A/T; and Y= C/T)

a) Heavy-chain VH

5'-primers (back primers):

VH1b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG CAG GAG
TCS G-3' [SEQ ID NO: 14]

VH2b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTA CAG CTG CAG CAG
TCA-3' [SEQ ID NO: 15]

VH3b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTA CAG CAG
TGG G-3' [SEQ ID NO: 16]

VH4b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC GAG GTG CAG CTG KTG GAG
WCY-3' [SEQ ID NO: 17]

VH5b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTC CAG CTK GTR CAG
TCT GG-3' [SEQ ID NO: 18]

VH6b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG RTC ACC TTG AAG GAG
TCT G-3' [SEQ ID NO: 19]

VH7b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG GTG SAR
TCT GG-3' [SEQ ID NO: 20]

3'-primers (forward primers):

VH1'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT
ATA CGA AGT TAT TGA TCC ACC ACC GCC TGA GGA GAC RGT GAC
CAG GGT G-3' [SEQ ID NO: 48]

VH2'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT
ATA CGA AGT TAT TGA TCC ACC ACC GCC TGA GGA GAC GGT GAC
CAG GGT T-3' [SEQ ID NO: 49]

VH3'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT
ATA CGA AGT TAT TGA TCC ACC ACC GCC TGA AGA GAC GGT GAC
CAT TGT-3' [SEQ ID NO: 50]

VH4'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT
ATA CGA AGT TAT TGA TCC ACC ACC GCC TGA GGA GAC GGT GAC
CGT GGT CC-3' [SEQ ID NO: 51]

VH5'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT
ATA CGA AGT TAT TGA TCC ACC ACC GCC GGT TGG GGC GGA TGC
ACT CC-3' [SEQ ID NO: 52]

VH6'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT
ATA CGA AGT TAT TGA TCC ACC ACC GCC SGA TGG GCC CTT GGT
GGA RGC-3' [SEQ ID NO: 53]

b) Light-chain V λ

5'-primers (back primers):

V λ 1'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT CAG TCT GTS BTG ACG
CAG CCG CC-3' [SEQ ID NO: 54]

V λ 2'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT TCC TAT GWG CTG ACW
CAG CCA C-3' [SEQ ID NO: 55]

V λ 3'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT TCC TAT GAG CTG AYR
CAG CYA CC-3' [SEQ ID NO: 56]

V λ 4'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT CAG CCT GTG CTG ACT
CAR YC-3' [SEQ ID NO: 57]

V λ 5'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT CAG DCT GTG GTG ACY
CAG GAG CC-3' [SEQ ID NO: 58]

Vλ6'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT CAG CCW GKG CTG ACT
CAG CCM CC-3' [SEQ ID NO: 59]

Vλ7'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT TCC TCT GAG CTG AST
CAG GAS CC-3' [SEQ ID NO: 60]

Vλ8'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT CAG TCT GYY CTG AYT
CAG CCT-3' [SEQ ID NO: 61]

Vλ9'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT AAT TTT ATG CTG ACT
CAG CCC C-3' [SEQ ID NO: 62]

3'-primers (forward primers):

Vλ1'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TAG GAC
GGT SAS CTT GGT CC-3' [SEQ ID NO: 63]

Vλ2'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT GAG GAC
GGT CAG CTG GGT GC-3' [SEQ ID NO: 64]

c) Light-chain Vκ

5'-primers (back primers):

Vκ1'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT GAC ATC CRG DTG ACC
CAG TCT CC-3' [SEQ ID NO: 65]

Vκ2'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT GAA ATT GTR WTG ACR
CAG TCT CC-3' [SEQ ID NO: 66]

Vκ3'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT GAT ATT GTG MTG ACB
CAG WCT CC-3' [SEQ ID NO: 67]

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SEQUENCE LISTING

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Hua, Shaobing

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<210> 10

<211> 34

<212> DNA

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<210> 11

<211> 34

<212> DNA

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<223> Description of Artificial Sequence: LoxP8

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<210> 12

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<223> Description of Artificial Sequence: LoxP9

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<210> 13
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<212> DNA

<213> Artificial Sequence

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<213> Artificial Sequence

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tttgatctcc ascttggtcc 80

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gcctgaggag acggtgacca gggtt 85

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<211> 84

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 50

actgcctcca cctgataact tcgtatagca tatattatac gaagttattg atccaccacc 60
gcctgaagag acggtgacca ttgt 84

<210> 51

<211> 86

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 51

actgcctcca cctgataact tcgtatagca tatattatac gaagttattg atccaccacc 60
gcctgaggag acggtgaccg tgggcc 86

<210> 52

<211> 83

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 52

ggcgggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agttcctatg agctgayrca gcyacc 86

<210> 57

<211> 83

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 57

ggcgggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agtcagcctg tgctgactca ryc 83

<210> 58

<211> 86

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 58

ggcgggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agtcagdctg tggtgacyca ggagcc 86

<210> 59

<211> 86

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 59

ggcgggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agtcagccwg kgctgactca gccmcc 86

<210> 60

<211> 86

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 60

ggcggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agttcctctg agctgastca ggascc 86

<210> 61

<211> 84

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 61

ggcggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agtcagtctg yyctgaytca gcct 84

<210> 62

<211> 85

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 62

ggcggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agtaatttta tgcagactca gcccc 85

<210> 63

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 63

cttcgtataa tgtatgctat acgaagttat taggaacggtc ascttggtcc 50

<210> 64

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer

<400> 64

cttcgtataa tgtatgctat acgaagttat gaggacggtc agctgggtgc 50

<210> 65
<211> 86
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 65
ggcgggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agtgacatcc rgdtgaccca gtctcc 86

<210> 66
<211> 86
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 66
ggcgggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agtgaaattg trwtgacrca gtctcc 86

<210> 67
<211> 86
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 67
ggcgggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agtgatattg tgmtgacbca gwctcc 86

<210> 68
<211> 85
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 68
ggcgggtggtg gatcaataac ttcgtataat atatgctata cgaagttatc aggtggaggc 60
agtgaaacga cactcacgca gtctc 85

<210> 69
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 69
cttcgtataa tgtatgctat acgaagttat ttgatttcc accttggtcc 50

<210> 70
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 70
cttcgtataa tgtatgctat acgaagttat ttgatctcc ascttggtcc 50

<210> 71
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 71
cttcgtataa tgtatgctat acgaagttat ttgatatcc actttggtcc 50

<210> 72
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 72
cttcgtataa tgtatgctat acgaagttat tttaatctcc agtcgtgtcc 50

<210> 73
<211> 78
<212> DNA

[illegible]

<223> Description of Artificial Sequence: PCR primer.

gagatggtgc acgatgcaca gttgaagtga acttgcgggg tttttcagta tctacgataa 60
cttcgtataa tgtatgct 78

<211> 63

<212> DNA

<213> Artificial Sequence

<223> Description of Artificial Sequence: Suc 2 signal

atgcttttgc aagcttttct tttctttttg gctggttttg cagccaaaat atctgcatca 60
atg 63

<211> 20

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: Linker peptide

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser
20